REMARKS

Reconsideration and continuing examination of the above-identified application is respectfully requested in view of the amendments above and the discussion that follows. Claims 1-47 are in the case and are currently under examination.

I. The Amendments

Claims 1, 25, and 47 have been amended to show more congruence with the specification. Support for these amendments can be found in paragraphs [0231]-[0234]. There is no new matter.

More specifically, the claims have been amended to more clearly state that the amino acid substitutions in the chimer are conservative, thereby preserving the structural and functional integrity of the molecule. Specific support for these amendments can be found in the specification as detailed below.

Notably, the MPEP (8th revision) states in section 2163, page 175;

> While there is no *in haec* requirement, newly added claim limitations must be supported in the specification through express, implicit, or inherent disclosure.

It is first mentioned that in the "Definitions" section of the present application, paragraph [0081] of the published application 2004/0146524 A1;

> The term 'corresponds' in its various grammatical forms as used in relation to peptide sequences means the peptide sequence described plus or minus up to about three amino acid residues at

> either or both of the amino- and carboxy-termini and containing only *conservative* (emphasis added) substitutions in particular amino acid residues along the polypeptide sequence.

Furthermore, in paragraph [0231] of the published application, the specification relates that:

> [A] contemplated chimer molecule can also contain *conservative* (emphasis added) substitutions in the amino acid residues that constitute HBC domains I, II, III, and IV.

In addition, paragraph [0232] of the published application, the specification relates:

> Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological activity or particle formation can be found using computer programs well known in the art, for example, LASERGENE software (DNASTAR Inc., Madison, Wis.).

Also, in paragraph [0233] of the applicaiton it is stated;

> When the HBc portion of a chimer molecule of the present invention *as above described* (emphasis added) has other than a sequence of a mammalian HBc molecule *corresponding* (emphasis added) to positions 2 through 183, up to about 20 percent of the amino acid residues are substituted as compared to SEQ ID NO:1 from positions 2 through 183, and preferably position 2 through 163. It is preferred that up to about 10 percent, and more preferably up to about 5 percent, and most preferably up to about 3 percent of the amino acid residues are substituted as compared to

> SEQ ID NO:1 from positions 2 though 163.

Still further, the published application states at paragraph [0234]:

> Substitutions, other than in the immunodominant loop of Domain II or at the termini, are preferably in the non-helical portions of the chimer molecule and are typically between residues 2 to about 15 and residues 24 to about 50 to help assure particle formation. See Koschel et al., (1999) *J. Virol.*, 73(3): 2153-2160.

The above passages illustrate that the specification describes and enables chimeric HBc molecules with conservative amino acid substitutions that retain biological activity and structural integrity. Based upon these passages, one of skill in the art would be able to make such HBc chimers. The specification points out that the amino acid substitutions are to be up to 5 percent of the sequence, that the substitutions are to be conservative, that guidance as to proper substitutions can be obtained through LASERGENE software and the like, and that the region of substitution is in the non-helical portion of the molecule, within residues 2-15 and 24-50, to assure particle formation. It is thus seen that no new matter has been added.

II. <u>The Action</u>

A. Claim Rejections

1. Rejections under 35 USC §112, First Paragraph

Claims 1-47 were rejected under 35 USC §112, First Paragraph, because allegedly, the specification while being enabling for a HBc chimer of SEQ ID NO:1, does not reasonably provide enablement for a HBc chimer containing up to about 5% substituted amino acid residues in the HBc SEQ ID NO:1. This basis for rejection is respectfully traversed in view of the above discussion of the amendments. Therefore, it is respectfully requested that this rejection be withdrawn.

2. Rejections under Doctrine of Obviousness-Type Double Patenting

The Action asserted that claims 1-46 were rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over (1) claims 1-78 of 09/930,915; (2) Claims 1-53 of 10/787,734; (3) Claims 98-109 of 10/805,913; (4) Claims 79-115 of 10/806,006; and (5) Claims 47-58 of 11/508,655.

The Examiner's comments about obviousness-type double patenting are noted. However, inasmuch as no claims of any of the applications cited in the Action are noted to be allowable, let alone allowed, it is still believed to be premature to deal with a terminal disclaimer.

It is respectfully requested that this rejection now be withdrawn.

3. Rejections under 35 USC §103(a)

a. First Rejection

Claims 1-6, 8-14, 16-28, 30-42 and 46 were again rejected under 35 USC §103(a) as being unpatentable over Pumpens in view of Zlotnick and Zheng. This rejection is again respectfully traversed.

The Action first discusses the alleged contribution of the Zlotnick manuscript. The premise that Zlotnick teaches that a C-terminal cysteine stabilizes HBcΔ still cannot be agreed with. The Zlotnick paper is lacking adequate controls needed to reach this conclusion. One of skill in the art would realize this.

The Action suggests that Zlotnick teaches that Cp*150 is more stable than the other molecules that Zlotnick made, namely, Cp149 and Cp*149. Cp149 is the 149-mer capsid protein. Cp*149 is the 149-mer capsid protein with the cysteines at positions 47, 61, and 107 mutated to alanine. Cp*150 is the 150-mer capsid protein with the cysteines at positions 47, 61, and 107 mutated to alanine and the C-terminal arginine mutated to cysteine. In order to reasonably conclude that the C-terminal cysteine is the cause of the alleged added stability of the molecule, there must be an exact length molecule of the exact sequence with a different amino acid besides cysteine at the C-terminus to compare and contrast. Without such a control 150-mer capsid protein, it cannot be properly concluded that the C-terminal cysteine was the cause of the alleged stabilization of the molecule.

It is equally plausible that the removal and substitution of arginine with another amino acid at the C-terminus of that molecule has led to increased stability of that molecule. It is conceivable that the terminal arginine is destabilizing to the molecule, in the absence

of a proper control. Cp149 is not a proper control of Cp*150. The length is not the same. A proper control would be another Cp*150 where there is a conservative amino acid substitution at the C-terminus, such as lysine for arginine. A second essential control would be another Cp*150 where a non-conservative amino acid substitution at the C-terminus is made. These controls are essential to a believable argument that the alleged stabilization was due to the C-terminal cysteine and these controls are missing in the Zlotnick paper.

As such, it cannot be concluded by one of skill in the art, that the C-terminal cysteine substitution was responsible for alleged increased stabilization. It must be noted that Zlotnick was not looking for ways to increase stability of capsid particles and is probably why he chose not to issue such a conclusion without the studying the proper controls.

Also, the Action suggested that the citations of Zlotnick presented in the last response were incomplete. This statement is not agreed with. It cannot be denied that Zlotnick (page 9558, Results and Discussion) made the statement that:

> Purified Cp*149 and Cp*150 assemble into capsids under the same conditions as other Cp constructs (10, 15), with or without DTT. These capsids were indistinguishable by negative staining electron microscopy and sedimentation on sucrose gradients (data not shown).

This passage states that molecules without C-terminal cysteine (Cp*149) assemble into capsids *under the same conditions as other Cp constructs*, including Cp*150, which

has the C-terminal cysteine. In other words, Zlotnick states that it makes no difference whether or not the capsid has a C-terminal cysteine in terms of stability and assembling into capsids. Also, Zlotnick states that the capsids studied (i.e., Cp*150 with and Cp149 without C-terminal cysteine) were *indistinguishable*. This is the *first* statement Zlotnick makes in his Results and Discussion section, which one of skill in the art would appreciate as to importance.

Next the Action highlights several sentences from Zlotnick regarding disulfide bonding. Again, without the proper controls, it is impossible for one of skill in the art to believe the quoted statements of Zlotnick about allegedly stability. To reiterate, there is only a 150-mer C-terminal cysteine capsid protein and no 150-mer control capsid protein.

Lastly, Zlotnick states on page 9560, column two, first paragraph that:

> Other observations imply that the C termini also may influence assembly in more subtle ways. For instance, binding Au11 to Cp*150 induces assembly, though Au11 cannot crosslink subunits nor, because of its organic shell, coordinate C-terminal cysteines. Binding Au11 may cause small changes in the molecular surfaces, near the C termini, that dock together when dimers polymerize and, in this way, stimulate the assembly process.

One of skill in the art would understand that Zlotnick suggests here that something besides cysteine binding is important in the assembly of capsid molecules. Zlotnick states that the data show that Au cannot cross-link

subunits nor coordinate C-terminal cysteines, yet the binding of Au11 to Cp*150 induces capsid assembly. Therefore, one of skill in the art would not conclude that C-terminal cysteines are responsible for capsid stabilization, but rather the opposite, that they are not that important. All in all, the Zlotnick manuscript is not valid as support of the premise that C-terminal cysteines enhance stability because it lacks proper controls and therefore conclusions gleaned from it are suspect. Furthermore, Zlotnick himself suggests that other factors come into play regarding inducing capsid assembly and stability, such as changes in molecular surfaces.

The Action next discusses the alleged contributions of the Zheng manuscript. The assertion that Zheng teaches that Cys48 and Cys107 are not essential for native core particle formation is agreed with. The basis for this assertion is the Abstract of Zheng on page 9422, as mentioned in the previous argument regarding Zlotnick, above. However, it must be emphasized that Zheng teaches that *no* disulfide bonds are necessary for particle formation. For example, Zheng states:

> Each of the cysteines of HBcAg has been eliminated both singly and in combination. All the proteins were shown to have very similar physical and immunochemical properties. All assemble into essentially identical core particle structures. Therefore, disulfide bonds are not essential for core particle formation. (see page 9422, Abstract).

Importantly, Zlotnick also teaches that neither of the cysteines at positions 48, 61, and 107 are vital for

particle formation. His mutant where those cysteines were all replaced by alanines still formed particles identical to the native 183-mer particle. (see page 9558, column 2, paragraph 1).

As for Pumpens, this article is not mentioned in the discussion of claim rejections in the last Action though the rejection is maintained. It is agreed that the statement from the previous Action that Pumpens does not teach replacing one or both cysteines at position 48 and 107 by another residue and adding a C-terminal cysteine.

Therefore, because the combination of Zlotnick, Zheng and Pumpens does not teach that the modification of positions 48 and 107 of HBc and the addition of a cysteine at the C-terminus would increase stability, it is respectfully requested that this rejection be withdrawn.

b. Second Rejection

Claims 1-6, 8-28, 30-46 again were rejected under 35 USC §103(a) as being unpatentable over Page and Birkett both in view of Zheng. This rejection is again respectfully traversed.

First, it must be noted that Page describes hybrid HBc capsid molecules having the three or four arginine repeats at the C-terminus deleted. The molecules of the present invention do not have the arginine repeats removed. As such, whatever Page may teach is not relevant to the present molecules as they are significantly different.

Also, the Action supplies a quotation from Page that is misleading without taking into consideration the

other sentences in that same paragraph and on that same page. The disclosure a whole must be understood.

The supplied quotation from Page (page 2) states that retention of the C-terminal cysteine allows for formation of a disulphide bond, which in the native structure is important for the formation of a stable particle. However, this is not the entire story, merely a quote taken out of context, and, as such, should not carry much weight. Several pages later, Page also states that a cysteine can be placed from one to twenty residues from the C-terminus (see lines 9-12, page 7).

The Page patent application states on page 2, lines 11-15, that the problematic binding of nucleic acid to HBcAg is known to be associated with four arginine repeats found at the C-terminus of the molecule. Page also states that removal of these arginine-DNA binding repeats is feasible and results in particles that do not bind DNA. However, Page states that removal of this region appears to reduce the inherent stability of the particle structure. Please note that the capsids of the present invention are not inherently unstable and do not have the arginine repeats removed.

Generalizations such as that which enhances one molecule also enhances another cannot be made if the molecules are dissimilar, especially in terms of stability and structure. It should be noted that the molecules of the present invention, besides retaining the arginine repeats also have the cysteines removed at positions 48 and 107. As presented in the Action, page 3, "a single amino acid can create problems resulting from changes in conformation that can't be adequately predicted in advance.

See Rudinger, J.". Following this line of thought, apples are not being compared to apples and generalizations regarding stability and lack thereof cannot be made with regard to capsid molecules with significant differences in amino acid sequence and therefore topology/structure.

Also Page does not teach that the replacement of cysteines 48 and 107 enhance the stability of the arginine repeat-containing capsid and does not teach the addition of a terminal cysteine to *such a capsid* results in added stability as well.

Although, Birkett is not mentioned in the Action, it must be noted that Birkett does not teach replacing one or both cysteines at positions 48 and 107, nor does Birkett address the added stability of such capsids with C-terminal cysteines.

Zheng teaches that no cysteines are important for capsid particle formation, as described in the previous argument. In addition, Zheng states on page 9426, first paragraph:

> The fact that all of the proteins were obtained as core particles clearly demonstrates that particle formation is not dependent on 1) the arginine-rich carboxyl-terminal domain of 22-kDa HBcAg(2), the binding of nucleic acid (3), or the formation of disulfide bonds.

It also must be noted that Zheng, nor any of the other papers cited, teaches that the removal of the cysteines at positions 48 and 107 enhances stability of the molecule. In fact quite the opposite is true. Zheng writes on page 9426, second column, third paragraph:

> Although Cys107 is a free sulfhydryl, it is not reactive under nondenaturing conditions. This suggests that this residue is either within the interior of the particle or rendered nonreactive by local conformational restrictions.

This passage does not suggest that somehow Cys107 is destabilizing to the molecule and therefore its removal would stabilize the molecule as the present invention has shown but that the presence of Cys107 has no effect.

The matter is less clear for Cys48 however. Zheng relates on page 9426, last paragraph:

> At present we have no explanation for the presence of both free and disulfide-bonded Cys48. Even prolonged exposure to oxidizing conditions (air, at pH 7-8.5) does not result in the conversion of all of the Cys48 to a disulfide. Perhaps, in the overall particle structure, only 50% of the Cys48 have the appropriate spatial relation to allow for such a bond. It is possible that the remaining free Cys48 could function in interaction with one of the 14 cysteines of the surface protein of HBV (HBcAg) in the assembly of the virus.

Here Zheng seems to suggest that Cys48 plays some role in assembly of the virus and as such may be important to the stability of the capsid molecule.

Therefore, the present invention as a whole was not *prima facie* obvious to one of ordinary skill in the art based on the combination of these three manuscripts as alleged in the Action. It is respectfully requested that this rejection be withdrawn.

B. Summary

Claims 1, 25 and 47 have been amended. Each of the bases for rejection has been dealt with and overcome or otherwise made moot.

It is therefore believed that this application is in condition for allowance of all of the pending claims. An early notice to that effect is earnestly solicited.

No further fee or petition is believed to be necessary. However, should any further fee be needed, please charge our Deposit Account No. 23-0920, and deem this paper to be the required petition.

The Examiner is requested to phone the undersigned should any questions arise that can be dealt with over the phone to expedite this prosecution.

Respectfully submitted,

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Enclosure
Petition and Fee
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